Psychopharmacology
DOI 10.1007/s00213-008-1448-5

ORIGINAL INVESTIGATION

Tamoxifen disrupts consolidation and retrieval of morphine-associated contextual memory in male mice: interaction with estradiol

Behnaz Esmaeili · Zahra Basseda · Shervin Gholizadeh · Mehrak Javadi Paydar · Ahmad Reza Dehpour

Received: 5 August 2008 / Accepted: 19 December 2008

Abstract
Rationale Tamoxifen (TMX), a selective estrogen receptor modulator, can affect cognitive functions of the brain. The conditioned place preference (CPP) paradigm involves memory for the association between contextual cues and the rewarding properties produced by a drug.
Objectives The effects of TMX alone and in combination with estradiol (E2) on reward-related memory of morphine were investigated in adult male mice.
Materials and methods Using an unbiased CPP paradigm, the ability of morphine sulfate (0.5–10 mg/kg, s.c.) to produce CPP was studied. Afterwards, the effects of TMX (1–10 mg/kg, s.c.) on the acquisition, consolidation, and expression of morphine-induced CPP were assessed. We have also evaluated the possible effects of s.c. E2 (10–200 μg/kg) and its co-administration with TMX (10 mg/kg, s.c.) on the consolidation and retrieval of morphine-associated contextual memory.
Results (1) Morphine (0.5–10 mg/kg) significantly induced CPP in a dose-dependent manner. (2) TMX (10 mg/kg) significantly reduced the time spent by mice in the morphine compartment when given immediately after each conditioning session (consolidation) or 30 min before testing for place preference in the absence of morphine (expression), whereas it had no effect when administered 30 min before each training session (acquisition). (3) Post-training or pre-testing administration of E2 increased morphine-induced CPP in a dose-dependent manner. (4) In addition, concomitant administration of E2 with TMX appears to prevent the impairing effect produced by TMX.
Conclusions TMX appears to disrupt consolidation and retrieval of morphine-associated contextual memory and this impairing effect might be prevented by E2 treatment.

Keywords Memory · SERM · Tamoxifen · Estradiol · Morphine · Conditioned place preference · Mice

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Introduction

The conditioned place preference (CPP) paradigm has been commonly used to study the reinforcing properties of various drugs including opioids (Tzschentke 1998; Bardo and Bevins 2000). This model is a type of Pavlovian conditioning that refers to a preference for an environment because of the contiguous association between the environmental stimuli (conditioned stimulus) and primary reinforcers (unconditioned stimulus). Therefore, specific environmental cues during drug administration can acquire incentive motivational effects that may provoke drug

Published online: 31 January 2009

craving and may contribute to relapse (Childress et al. 1988). Since expression of CPP is inferred in a drug-free state, CPP behavior involves reward-related memory for an association between contextual cues and the emotional state produced by a drug (White and Carr 1985; Schroederl and Packard 2003).

Estrogen receptor (ER) is expressed in different brain regions and mediates a variety of functions in the nervous system, including pain mechanisms, fine motor skills, mood, reward, cognition, and affect (McEwen 2001). Administration of estradiol (E2) that results in physiological E2 levels in the nucleus accumbens of ovariectomized rats produces a CPP (Frye and Rhodes 2006). Female rats showed cocaine-induced place preference at lower doses than male rats and ovarian hormones appeared to be responsible for this greater sensitivity to cocaine's reinforcing effects (Russo et al. 2003a, b). E2-treated female rats showed an augmented amphetamine-induced place preference compared to vehicle-treated or ovariectomized controls (Silverman and Koenig 2007). Numerous studies have shown that estrogen exerts important effects on cognitive functions of the brain (Farr et al. 1995; Packard and Teather 1997; Rissanen et al. 1999). However, these effects of estrogen are varied from augmentation of cognitive performance in some learning and memory-dependent paradigms to impairment of cognitive performance in others. Estrogen-treated ovariectomized female mice showed improved spatial memory in a water maze paradigm and non-spatial memory in passive avoidance and object recognition models (Heikkinen et al. 2002; Rissanen et al. 1999; Farr et al. 1995; Gresack and Frick 2004). However, some studies report that estrogen impaired or had no effect on memory-dependant tasks. For example, using Morris water maze, gonadally intact female rats and mice showed longer escape latencies to find the hidden platform compared to ovariectomized controls (Frye 1995; Warren and Juraska 1997; Wilson et al. 1999). In the radial arm maze task, estrogen given to ovariectomized rats had no effect on reference memory (Fader et al. 1999).

Tamoxifen (TMX) is a selective estrogen receptor modulator (SERM) that has been shown to have agonist- and antagonist-like effects on ER in different brain regions (Diel 2002; Halbreich and Kahn 2000). TMX has demonstrated neuroprotective effects similar to estrogen on nigrostriatal dopaminergic neurons (Dluzen and McDermott 2002; Dluzen 2000). It also has agonist estrogenic activity on brain *N*-methyl-D-aspartate (NMDA) and AMPA receptors (Cyr et al. 2001). In some conditions, TMX attenuates the effects of estrogen; Walf and Frye (2005) have shown that TMX reduces anxiolytic effects of estrogen in rats. However, some studies have reported no interaction between TMX and estrogen effects; systemic TMX failed to attenuate systemic or intrahippocampal E2's significant enhancing effect on inhibitory avoidance performance (Frye and Rhodes 2002). TMX impaired learning and memory abilities in passive avoidance behavioral task in intact male mice independent of E2 (Chen et al. 2002a); it also impaired the retrieval of spatial information in Morris water maze (Chen et al. 2002b). Besides these experimental studies, there are some clinical reports on the memory-impairing effects of adjuvant chemotherapy of breast cancer with TMX (Bender et al. 2006; Castellon et al. 2004; Jenkins et al. 2004; Eberling et al. 2004; Shilling et al. 2003; Falleti et al. 2005). Previous studies evaluating the effect of TMX on the brain reward system have shown that TMX attenuates the effects of E2 on cocaine self-administration (Lynch et al. 2001); also, E2-induced place preference has been diminished in the presence of TMX (Walf et al. 2007). Despite these modulatory effects on the reward system, TMX alone does not show any rewarding or aversive properties (Walf et al. 2007).

The impairing effects of TMX on learning and memory in some circumstances and its mentioned modulatory effects on the reward system raise this question whether TMX has some effects on the reward system and/or cognitive processes involved in reward-evaluating models. Chen et al. (2002a) have shown that TMX affects cognitive function in intact male and female mice regardless of sex difference. In addition, using place preference paradigm, we previously found that there is no significant difference between intact male and female mice to show rewarding or aversive properties of TMX (unpublished data). A previous study reported no differences in spatial working memory between intact male and female rats (Healy et al. 1999). In addition, some studies have shown that ovariectomy of female mice and rats can affect cognitive performance (El-Bakri et al. 2004; Heikkinen et al. 2002); thus, we used male mice to avoid the potential influence of female hormones on cognitive processes. The present studies were designed to assess the dose-responsive effects of morphine on acquisition of CPP (Exp 1), elucidate the effects of tamoxifen on the acquisition (Exp 2), consolidation (Exp 3), and expression (Exp 4) of morphine-induced CPP independent of E2. In addition, the effects of E2 to modulate the effects of tamoxifen on consolidation (Exp 5) and expression (Exp 6) of morphine-induced CPP were also investigated.

Materials and methods

Animals

Male NMRI (a commonly used strain) mice (Institute Pasteur of Iran, Tehran, Iran), weighing 20–30 g were used. The animals were housed seven to nine per cage in a temperature-controlled (22±3°C) colony room. They were

maintained in a 12-h on and 12-h off light/dark schedule with ad libitum food and water, except during experimental procedures. All experiments were conducted between Zeitgeber Time (ZT) 3 and ZT 5 (light onset is defined as ZT 0). Subjects were experimentally naive. Animals were allowed 7 days to acclimatize to the laboratory environment before testing began. Each mouse was used only once and each treatment group consisted of seven to nine animals. Animals housed in the same cage were subjected to the same treatment. All procedures were carried out in accordance with institutional guidelines for animal care and use, and possible measures were undertaken to minimize the number of animals used and also to minimize animals' discomfort. The protocol was approved by the Committee of Ethics of the Faculty of Sciences of Tehran University (357; 8 November 2000).

Drugs

In the present study, all mice were administered either morphine sulfate (Temad Pharmaceutical, Tehran, Iran) or saline vehicle. Dosages of morphine used in the present study (0.5, 2.5, 5, and 10 mg/kg) were based upon previous studies indicating that some dosages in this range will reliably elicit CPP (Esmaeili et al. 2008; Tahsili-Fahadan et al. 2006). In addition, mice were administered either tamoxifen citrate (0.5% dimethylsulfoxide (DMSO)–sesame oil *v*/*v*; Iran Hormone Co., Tehran, Iran) or DMSO–sesame oil vehicle. In experiments investigating estradiol's effects, mice were administered either estradiol benzoate (Abureyhan Pharmaceutical, Tehran, Iran) or sesame oil vehicle. The dosages and timing of administrations of tamoxifen and E2 in the present study were based upon prior reports (Chen et al. 2002a; Walf et al. 2007). All drugs were injected subcutaneously (s.c.) in a volume of 5 ml/kg.

Place conditioning apparatus

The place preference apparatus were made of wood, consisted of two square-based compartments (15 cm× 15 cm×30 H cm each) with different visual and sensory textures, smooth and grid. The inner surface of smooth compartment was painted black and the grid one was white to create equally preferred compartments. During the conditioning phase, two compartments were separated by a guillotine door and covered with a transparent Plexiglas ceiling (Groblewski et al. 2008; Tahsili-Fahadan et al. 2006). The time spent in each compartment was recorded via a stopwatch. To measure the locomotor activity, the ground areas of the compartments were divided into four equal-sized squares (7.5×7.5 cm), and the number of mice entrance to each square was recorded by another observer and used as an index of locomotor activity (Langroudi et al. 2005; Tahsili-Fahadan et al. 2006).

Measurement of CPP

Conditioned place preference was conducted using an unbiased procedure (Tahsili-Fahadan et al. 2006; Esmaeili et al. 2008). It consisted of a 9-day schedule with three distinct phases: familiarization and pre-conditioning, conditioning, and post-conditioning.

Familiarization and pre-conditioning

On the first (i.e., familiarization) and second (i.e., pre-conditioning) trial days, mice were individually placed into the apparatus for 10 min, during that time they could freely access both compartments. The time spent in each compartment was recorded on the pre-conditioning day to determine any individual innate preference for each compartment. Placement in each compartment was defined as placement of the front paws and the head. Animals showing strong unconditioned preference for any compartment (i.e., time spent in each compartment > mean+2SD) were excluded from the experiments (a total number of four mice). Following pre-conditioning, mice were randomly assigned to receive morphine in one compartment and saline in the other during the conditioning phase.

Conditioning

This phase consisted of six consecutive conditioning sessions (1/day), each 40 min in length (Langroudi et al. 2005; Tahsili-Fahadan et al. 2006). Mice were confined to the considered compartment, by isolating the compartment using a removable partition. The mice received morphine on days 1, 3, and 5, and saline on days 2, 4, and 6 of the conditioning phase immediately prior to placement into the apparatus. Treatment compartment and order of presentation of drugs and saline were counterbalanced for either group.

Post-conditioning

This phase was carried out on the ninth day of the trials (24 h after the last conditioning session, with no preceding injections) in a drug-free state. As in the pre-conditioning phase, the partition was raised and the animals were placed in the apparatus for 10 min, with free access to both compartments and the time spent in each compartment was recorded in real time by an observer blind to treatments and groups. Change in preference (CIP) was calculated as the difference (in seconds) between the time spent in the drug-paired compartment on the post-conditioning day and the time spent in this compartment in the pre-conditioning session.

Measurement of locomotor activity

Locomotor testing was carried out in both conditioning sessions (for acquisition experiments) and post-conditioning test (for expression experiments) as proposed in a previous study (Meyers et al. 2006) with some modifications as mentioned previously.

CPP experimental design

Dose-response effects of place conditioning produced by morphine

Immediately prior to placement in the CPP apparatus, mice were administered 0 (n=7), 0.5 (n=7), 2.5 (n=7), 5 (n=7), or 10 (n=7) mg/kg of morphine on the appropriate days of conditioning. On alternate days, all mice received saline injections before placement in the apparatus. Mice were tested in a morphine-free state.

Effect of pre-treatment with tamoxifen during conditioning sessions on the acquisition of morphine-induced CPP

Thirty minutes prior to placement in the CPP apparatus, mice were administered 0 (n=8), 1 (n=8), 3 (n=8), or 10 (n=8) mg/kg TMX on each day of conditioning. Immediately prior to conditioning, mice were administered morphine (10 mg/kg) or saline. A control group administered DMSO–sesame oil vehicle and saline prior to conditioning was used to assess the effectiveness of morphine to induce CPP. Twenty-four hours after completion of the conditioning procedure, mice were tested for CIP.

Effect of post-treatment with tamoxifen during conditioning sessions on the consolidation of morphine-induced CPP

Mice were administered either morphine (10 mg/kg) or saline immediately prior to conditioning. TMX 0 (n=8), 1 (n=8), 3 (n=8), or 10 (n=8) mg/kg was administered within 3 min (referred as immediate) after completing each training session during conditioning phase. A control group administered saline in all conditioning sessions and DMSO–sesame oil vehicle after conditioning was used to assess the effectiveness of morphine to induce CPP. Following injection, mice were returned to their home cages in the colony.

Effect of pre-test injection of tamoxifen on the expression of morphine-induced CPP

Immediately prior to placement in the CPP apparatus, mice received morphine (10 mg/kg) on the appropriate days of conditioning. On alternate days, mice received saline injections before conditioning. On day 9, animals were tested in a morphine-free state. On day 10, mice that exhibited CPP on the test day were pretreated with 0 (n=8), 1 (n=8), 3 (n=8), or 10 (n=8) mg/kg TMX and after 30 min were re-tested for CPP. A control group received saline in all six training sessions and DMSO–sesame oil vehicle 30 min before the second test for CPP was used to assess the effectiveness of morphine to induce CPP. Two mice were excluded from this experiment because they did not show CPP on day 9.

Effect of post-treatment with estradiol and its combination with tamoxifen during conditioning sessions on the consolidation of morphine-induced CPP

Mice were administered E2 0 (n=9), 10 (n=9), 20 (n=8), 50 (n=9), 100 (n=9), or 200 (n=9) μg/kg within 3 min following completing the training session on each conditioning day. A control group was administered saline in all six conditioning sessions and E2's vehicle immediately after training. To investigate the effect of co-administration of TMX and E2 on the consolidation of the reward-related memory of morphine, E2 0, 10, 20, 50, 100, or 200 μg/kg (n=8–9, each group) in conjunction with TMX (10 mg/kg) or its vehicle was administered immediately following completing each training session. Following injection, mice were returned to their home cages in the colony.

Effect of pre-test injection of estradiol and its combination with tamoxifen on the expression of morphine-induced CPP

Mice that showed CPP on day 9 were administered E2 0 (n=9), 10 (n=9), 20 (n=8), 50 (n=9), 100 (n=9), or 200 (n=8) μg/kg 30 min before the second test for CPP on day 10. A control group was administered saline in all six conditioning sessions and E2's vehicle 30 min before the second test for CPP was used to assess the effectiveness of morphine to induce CPP. To study the effect of co-administration of TMX and E2 on the expression of morphine-induced CPP, E2 0, 10, 20, 50, 100, or 200 μg/kg (n=8–9, each group) was administered in conjunction with TMX (10 mg/kg) or its vehicle 30 min before the second test for CPP on day 10.

Data analysis

All results are presented as mean±SEM. Data for CIP and locomotion were assessed by one-way analysis of variance (ANOVA) or, when appropriate, two-way ANOVA. If a significant F value was obtained, post hoc analyses (Bonferroni's multiple comparison tests) were performed to determine the effects of various treatments on induction of place preference and changes in locomotion. P values

less than 0.05 were considered as significant. Calculations were performed using SPSS statistical package (version 11.5).

Results

Dose-response curve for place preference conditioning produced by morphine in mice

As shown in Fig. 1a, morphine dose-dependently produced a CPP (one-way ANOVA; $F(4, 34)=12.220$, $P<0.001$). Post hoc analyses revealed that the three highest doses of morphine (2.5, 5, and 10 mg/kg) produced a significant CPP compared to the lowest dose (0.5 mg/kg) and vehicle. The results did not show any significant effect for morphine

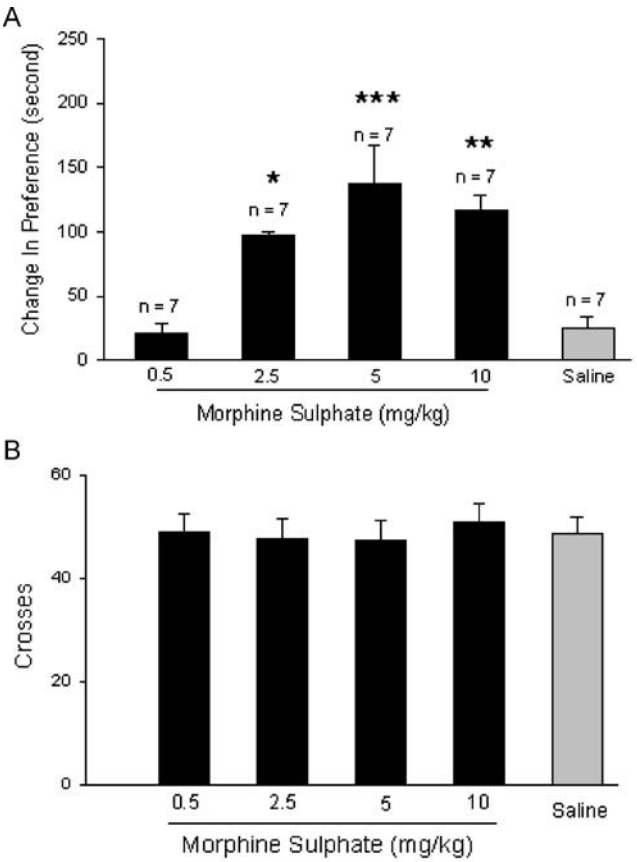

Fig. 1 Effect of morphine on (**a**) CPP induction in mice. Animals received saline (5 ml/kg, s.c.) or morphine (0.5–10 mg/kg, s.c.) in the drug-paired compartment on the first, third, and fifth days of conditioning. The data are shown as mean±SEM of change in preference (in seconds). *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared to the group treated with saline (Bonferroni's multiple comparison tests); (**b**) locomotor activity. The data are shown as mean±SEM of crosses. Analysis revealed that no group showed a statistical significant difference

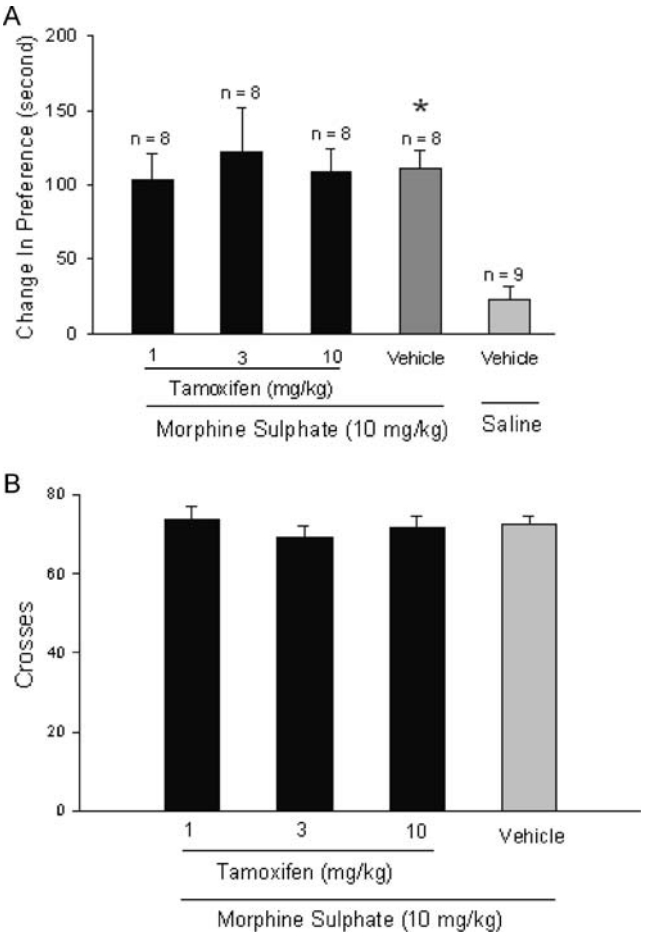

Fig. 2 Effect of pre-treatment with tamoxifen during conditioning sessions on (**a**) the acquisition of morphine-induced CPP. Values represent mean±SEM of change in preference (in seconds). Tamoxifen (1, 3, and 10 mg/kg) and its vehicle (5 ml/kg) were injected 30 min prior to all six conditioning sessions. All groups revealed significant preference for drug-paired compartment. *$P<0.05$ compared to the TMX's vehicle/saline control group (Bonferroni's multiple comparison tests); (**b**) locomotor activity. The data are shown as mean±SEM of crosses. Analysis revealed that no group showed a statistical significant difference

on locomotor activity in this experiment (one-way ANOVA; $F(4, 34)=0.158$, $P=0.958$; Fig. 1b).

Effect of pre-treatment with tamoxifen during conditioning sessions on the acquisition of morphine-induced CPP

As shown in Fig. 2a, pre-treatment with TMX during conditioning sessions had no significant effect on the acquisition of morphine-induced CPP (one-way ANOVA; $F(3, 31)=0.167$, $P=0.918$). All groups showed morphine-induced CPP compared to the saline/TMX's vehicle control group ($P<0.05$). The results did not show any significant effect for TMX on locomotor activity in this experiment (one-way ANOVA; $F(3, 31)=0.382$, $P=0.766$; Fig. 2b).

Effect of post-treatment with tamoxifen during conditioning sessions on the consolidation of morphine-induced CPP

As shown in Fig. 3, post-treatment with TMX produced a significant effect on morphine-induced CPP (one-way ANOVA; $F(3, 31)=5.986$, $P=0.003$). Post hoc analyses revealed that the highest dose of TMX (10 mg/kg) disrupted consolidation of morphine-induced CPP compared to the other doses of TMX (1 and 3 mg/kg) and vehicle.

Effect of pre-test injection of tamoxifen on the expression of morphine-induced CPP

As shown in Fig. 4a, pre-test injection of TMX produced a significant effect on morphine-induced CPP (one-way ANOVA; $F(3, 31)=6.667$; $P=0.002$). Post hoc analyses showed that TMX (10 mg/kg) disrupted retrieval of morphine-induced CPP compared to the other doses of TMX (1 and 3 mg/kg) and vehicle. The results did not show any significant effect for TMX on locomotor activity in this experiment (one-way ANOVA; $F(3, 31)=0.285$, $P=0.836$; Fig. 4b).

Effects of post-treatment with estradiol and its combination with tamoxifen during conditioning sessions on the consolidation of morphine-induced CPP

As shown in Fig. 5, post-treatment with E2 increased CIPs in a dose-dependent manner (one-way ANOVA; $F(5, 52)=3.315$; $P=0.01$). Post hoc analyses revealed that this increasing effect of E2 on morphine-induced place preference was not statistically significant compared to the morphine/E2's vehicle control group. All six groups showed significant CPP compared to the saline/E2's vehicle control group ($P<0.05$). As shown in Fig. 6, two-way ANOVA indicated a significant interaction between E2 and TMX on the consolidation of morphine-induced CPP (factor E2, $F(5, 102)=4.136$, $P<0.01$; factor TMX, $F(1,$

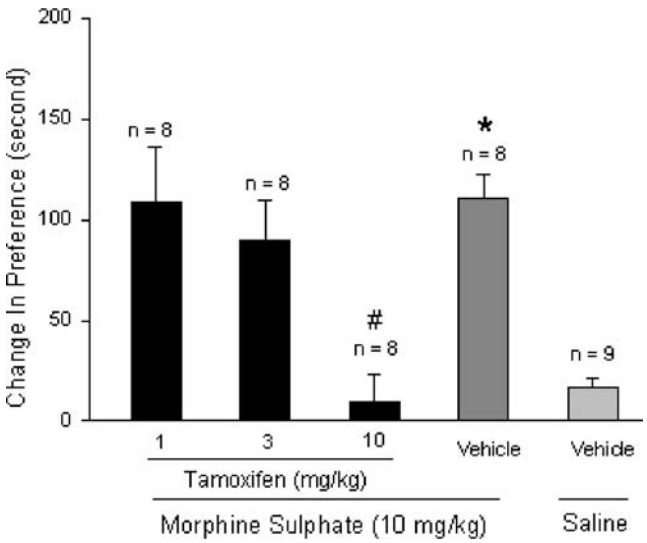

Fig. 3 Effect of post-treatment with tamoxifen on the consolidation of morphine-induced CPP. Values represent mean±SEM of change in preference (in seconds). Tamoxifen (1, 3, and 10 mg/kg) or its vehicle (5 ml/kg) was injected immediately following all six conditioning sessions. #$P<0.01$ compared to the group receiving morphine on the first, third, and fifth days of conditioning and post-treatment with TMX's vehicle; *$P<0.01$ compared to the saline/TMX's vehicle control group (Bonferroni's multiple comparison tests)

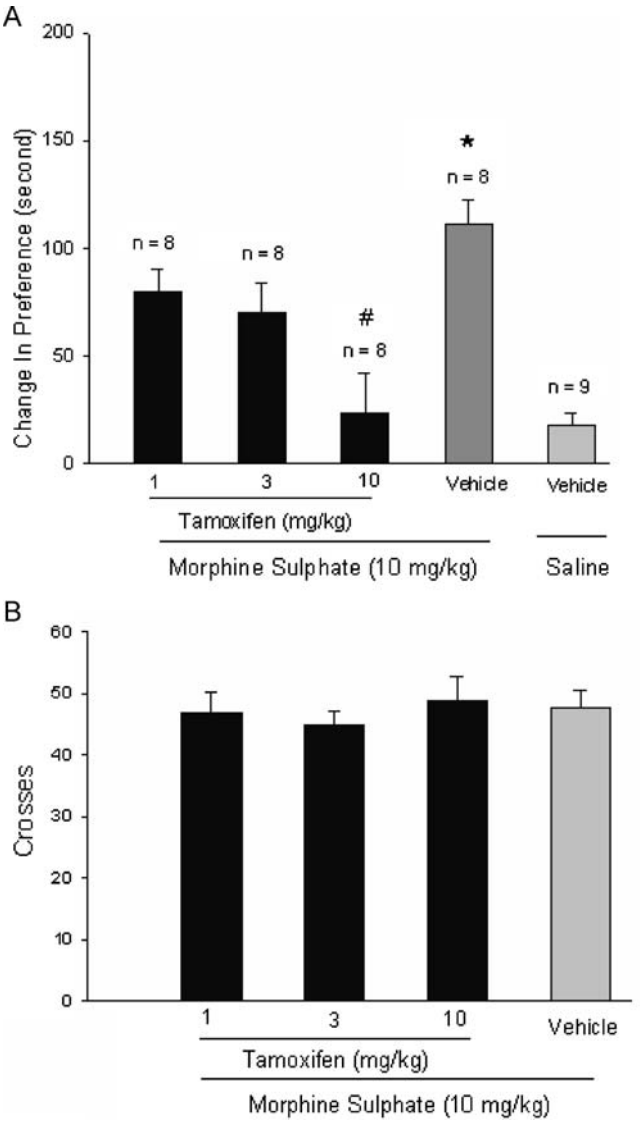

Fig. 4 Effect of pre-test injection of tamoxifen on (**a**) the expression of morphine-induced CPP. Values represent mean±SEM of change in preference (in seconds). Mice received tamoxifen (1, 3, and 10 mg/kg) or its vehicle (5 ml/kg) 30 min prior to testing for CPP. #$P<0.01$ compared to the group receiving morphine on the first, third, and fifth days of conditioning and pre-testing treatment with TMX's vehicle; *$P<0.001$ compared to the saline/TMX's vehicle control group (Bonferroni's multiple comparison tests); (**b**) locomotor activity. The data are shown as mean±SEM of crosses. Analysis revealed that no group showed a statistical significant difference

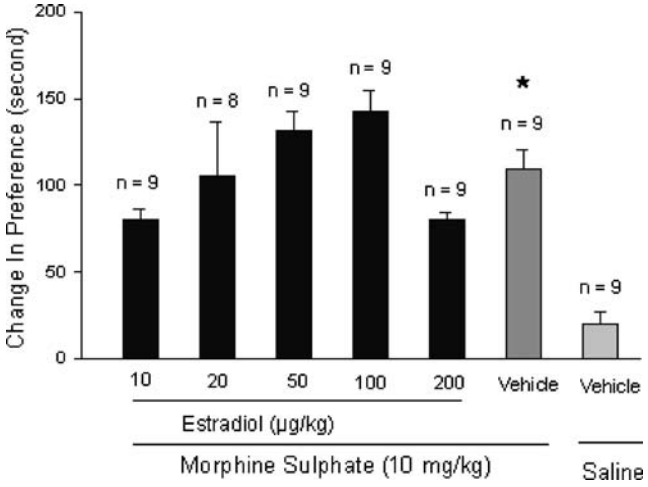

Fig. 5 Effect of post-treatment with estradiol on the consolidation of morphine-induced CPP. Values represent mean±SEM of change in preference (in seconds). Estradiol (10, 20, 50, 100, and 200 µg/kg) or its vehicle (5 ml/kg) was injected immediately following all six conditioning sessions. *P<0.001 compared to the group receiving saline injections in all six conditioning sessions and E2's vehicle immediately after each training session (Bonferroni's multiple comparison tests)

102)=25.007 , P<0.001; factor E2 × TMX, F(11, 102)= 6.341, P<0.001). Post hoc analyses showed that E2 decreased the impairing effect of TMX on the consolidation of morphine-induced CPP. Co-administration of E2 (100 µg/kg) with TMX (10 mg/kg) significantly decreased the impairing effect of TMX on the consolidation of reward-related memory of morphine compared to the E2's vehicle control group (P=0.001).

Effects of pre-test injection of estradiol and its combination with tamoxifen on the expression of morphine-induced CPP

As shown in Fig. 7a, pre-test injection of E2 increased CIPs in a dose-dependent manner (one-way ANOVA; F(5, 51)= 13.228; P<0.001). Post hoc analyses revealed that E2 (100 µg/kg) significantly increased the morphine-induced place preference compared to the morphine/E2's vehicle control group. All six groups showed CPP compared to the saline/E2's vehicle control group (P<0.001). The results did not show any significant effect of E2 on locomotor activity in this experiment (one-way ANOVA; F(5, 51)= 0.457, P=0.806; Fig. 7b). Two-way ANOVA indicated a significant interaction between E2 and TMX on the expression of morphine-induced CPP (factor E2, F(5, 103)=12.128, P<0.001; factor TMX, F(1, 103)=5.918, P=0.01; factor E2 × TMX, F(11, 103)=9.679, P<0.001; Fig. 8a). Post hoc analyses showed that co-administration of E2 (50, 100, and 200 µg/kg) with TMX (10 mg/kg) decreased the impairing effect of TMX on the retrieval of morphine-associated contextual memory (P<0.001, P< 0.001, and P<0.01, respectively in comparison with morphine/E2's vehicle/TMX control group). The results did not show any significant effect for TMX co-administered with E2 on locomotor activity in this experiment (one-way ANOVA; F(6, 61)=0.174, P=0.983; Fig. 8b).

Discussion

Tamoxifen is a selective estrogen receptor modulator (SERM) that has been shown to have agonist- and antagonist-like effects on ER in different brain regions. It has been shown that TMX affects different brain functions such as motor skills, anxiety, mood, reward, learning, and memory (McEwen 2001). Besides clinical reports (Bender et al. 2006; Castellon et al. 2004; Jenkins et al. 2004; Eberling et al. 2004; Shilling et al. 2003; Falleti et al. 2005) on the memory-impairing effects of adjuvant chemotherapy of breast cancer with TMX, some studies have demonstrated the impairing effect

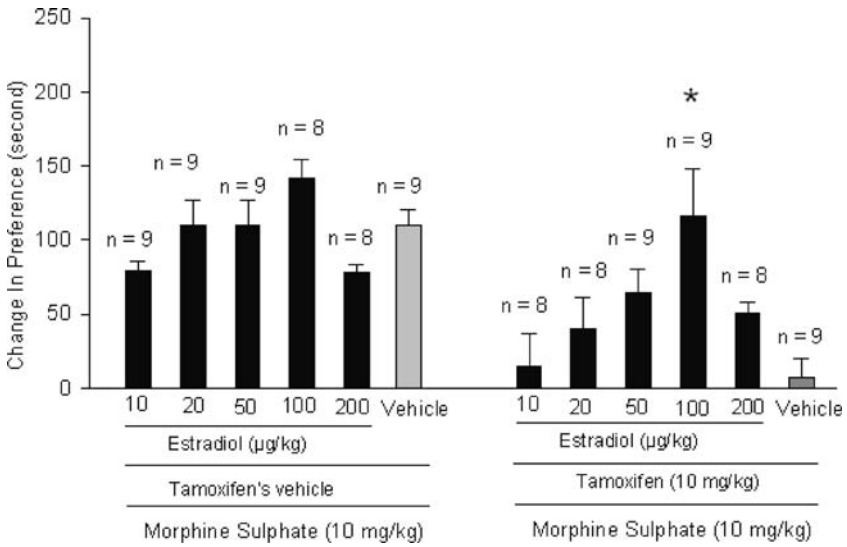

Fig. 6 Effect of post-treatment with estradiol in combination with tamoxifen on the consolidation of morphine-induced CPP. E2 (10, 20, 50, 100, and 200 µg/kg) or its vehicle (5 ml/kg) was co-administered with TMX (10 mg/kg) or its vehicle immediately after each conditioning session. The data are shown as mean±SEM of change in preference (in seconds). *P<0.01 compared to the morphine/E2's vehicle/TMX control group (Bonferroni's multiple comparison tests)

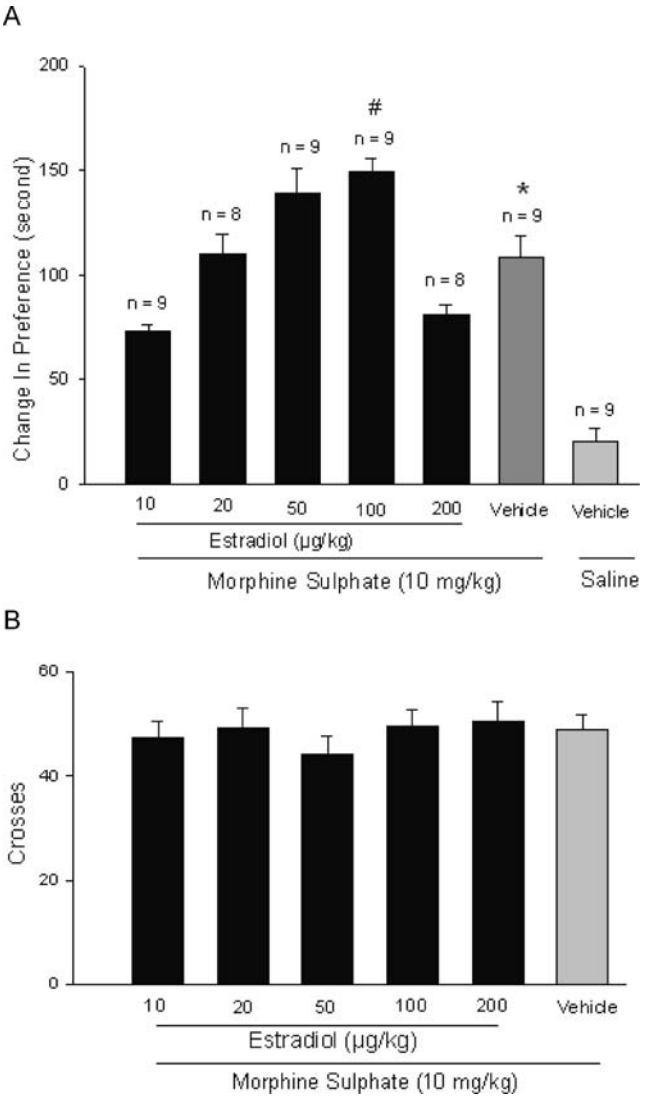

Fig. 7 Effect of pre-test injection of estradiol on (**a**) the expression of morphine-induced CPP. Values represent mean±SEM of change in preference (in seconds). Mice received estradiol (10, 20, 50, 100, and 200 µg/kg) or its vehicle (5 ml/kg) 30 min prior to testing for CPP. #$P<0.01$ compared to the group receiving pre-testing treatment with E2's vehicle; *$P<0.001$ compared to the saline/E2's vehicle control group (Bonferroni's multiple comparison tests); (**b**) locomotor activity. The data are shown as mean±SEM of crosses. Analysis revealed that no group showed a statistical significant difference

of this drug on cognitive functions in experimental animals (Chen et al. 2002a, b). TMX has also a modulatory effect on brain reward system. However, it has been shown that TMX by itself does not have any rewarding or aversive properties. It seems that TMX has an antiestrogenic effect on the reward system of the brain (Walf et al. 2007). The current study evaluates the effects of systemic administration of TMX on the rewarding properties of morphine. We also have investigated the possible effects of TMX and its co-administration with E2 on learning and memory processes underlying morphine-induced place preference.

Results showed that morphine induces a significant CPP in a dose-dependent manner in mice, which is consistent with previous studies (Tzschentke 1998). Drugs at the doses used in our experiments did not alter locomotor activity in comparison with the control groups. Pre-treatment with TMX during conditioning sessions, at least in the dose range used here (1, 3, and 10 mg/kg) did not interfere with the acquisition of morphine-induced CPP. A previous study (Walf et al. 2007) has reported that TMX alone does not show any significant effect on place conditioning; however, concomitant administration of TMX (10 mg/kg) with estradiol attenuates the effects of E2 to produce a CPP (Walf et al. 2007). It also attenuates E2's effects on cocaine self-administration (Lynch et al. 2001). Our findings showed that TMX does not appear to interfere with morphine-induced rewarding properties. Furthermore, it seems that TMX has no effect on cognitive processes underlying initial acquisition of learning in morphine-induced CPP. In this line, previous studies have reported that TMX has no effect on initial acquisition of other learning and memory-dependent behavioral tasks including passive avoidance and Morris water maze in mice (Chen et al. 2002a, b). The present results supported our hypothesis that TMX's modulatory effects on reward-dependent paradigms might be due in part to effects on memory processes underlying these paradigms.

Here, we demonstrate that s.c. TMX (10 mg/kg) administration immediately following each conditioning session appears to impair morphine-induced CPP, suggesting that the consolidation of morphine-associated contextual memory may be disrupted by post-training TMX administration. Another possible interpretation of this result is that TMX does not impair the memory for morphine-induced CPP but impairs the rewarding properties of morphine. However, in the present experimental design, mice received post-training TMX in association with both conditioning compartments; considering the algebric summation of hedonic processes (Young and Christensen 1962), if TMX had aversive properties per se, exposure to the saline compartment would be expected to be more aversive than exposure to the morphine compartment. Therefore, aversive properties of TMX should have little effect on the place preference. On the other hand, if TMX blocked the primary rewarding properties of morphine, pre-training TMX administration should have been more effective than its post-training administration. Moreover, our results showed that pre-testing treatment with TMX (10 mg/kg) appears to impair expression of morphine-induced CPP. It seems that TMX interferes with the retrieval of morphine-induced reward-associated memory when a CPP task is already well-learned. A putative interference on locomotor activity was excluded based on the results of measurement of locomotor activity in post-conditioning test, in which TMX

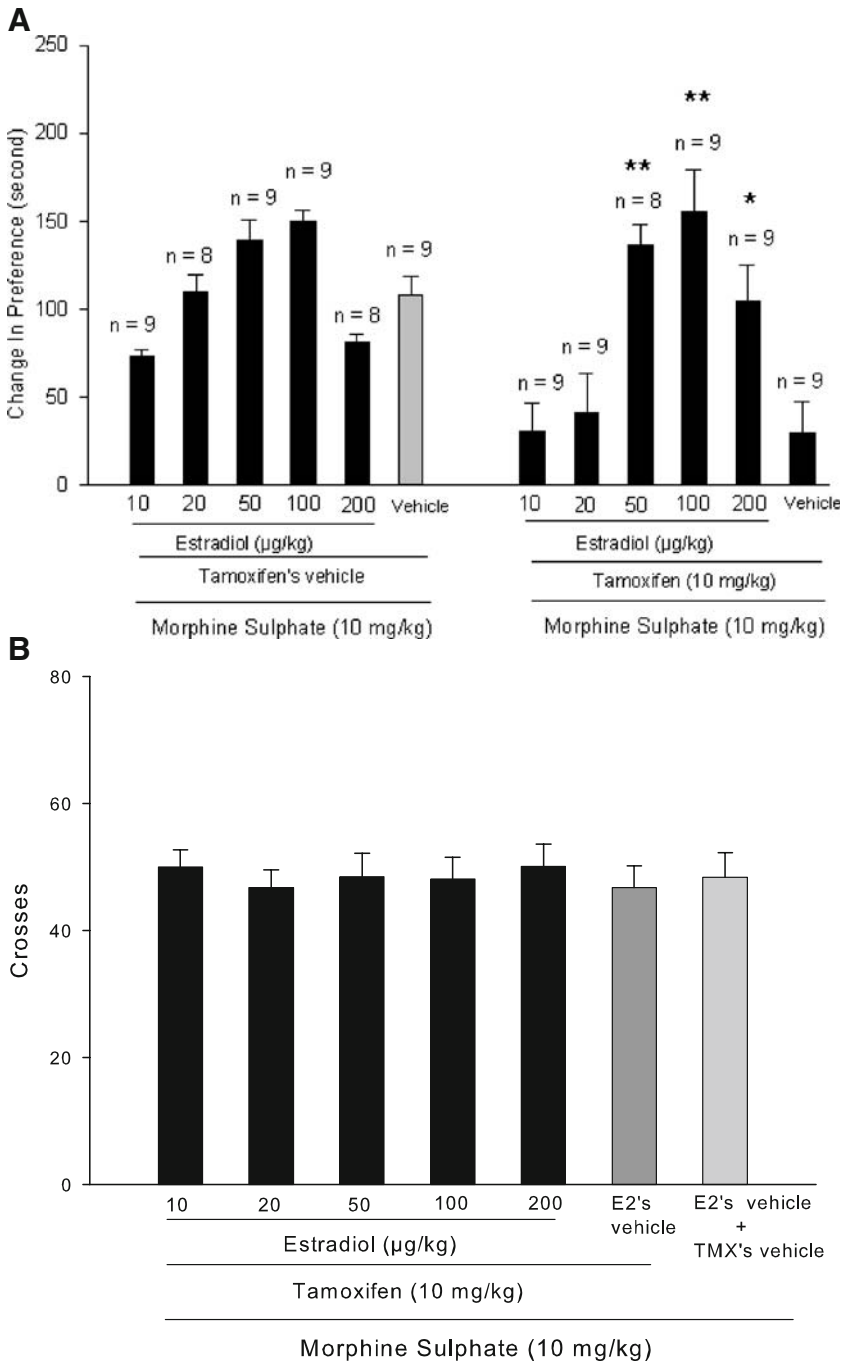

Fig. 8 Effect of pre-test co-administration of estradiol and tamoxifen on (**a**) the expression of morphine-induced CPP. E2 (10, 20, 50, 100, and 200 μg/kg) or its vehicle (5 ml/kg) was co-administered with TMX (10 mg/kg) or its vehicle 30 min before the second test for CPP. The data are shown as mean±SEM of change in preference (in seconds). *$P<$ 0.05, **$P<0.001$ compared to the morphine/E2's vehicle/TMX control group (Bonferroni's multiple comparison tests); (**b**) locomotor activity. The data are shown as mean±SEM of crosses. Analysis revealed that no group showed a statistical significant difference

administration did not affect the mice ambulation, suggesting that the impairment observed with pre-test administration of tamoxifen is due to an effect during retrieval of reward-related memory not on performance. These findings complement those from previous studies that demonstrate TMX impairs processes underlying memory consolidation and retrieval in passive avoidance and Morris water maze behavioral paradigms (Chen et al. 2002a, b).

Our results revealed that systemic E2 immediately following each conditioning session or 30 min before testing for the CPP appears to increase CIP in a dose-dependent manner in the place preference paradigm. However, only E2 (100 μg/kg) had a statistically significant increasing effect on the retrieval of morphine-induced contextual memory compared to the morphine/E2's vehicle control group. The present results extend previous reports that have shown estrogen can modulate cognitive performance (Farr et al. 1995; Packard and Teather 1997; Rissanen et al. 1999). The effects of E2 on cognitive functions are varied from augmentation of cognitive

performance in some cognitive tasks to impairment of cognitive performance in others. Ovariectomized female mice receiving estrogen treatment showed an improved spatial memory in a water maze paradigm and enhanced performance in object recognition and passive avoidance learning and memory-dependent models (Heikkinen et al. 2002; Rissanen et al. 1999; Farr et al. 1995; Gresack and Frick 2004). However, some studies report that estrogen impaired or had no effect on memory-dependent tasks. For example, using the Morris water maze, gonadally intact female rats and mice showed longer escape latencies to find the hidden platform compared to ovariectomized controls (Frye 1995; Warren and Juraska 1997; Wilson et al. 1999). In the radial arm maze task, estrogen given to ovariectomized rats had no effect on reference memory (Fader et al. 1999).

We also demonstrated that with increasing dose of E2, CIPs of mice receiving E2/TMX treatment post-training or before the second test for CPP were not significantly different from CIPs of mice receiving E2/TMX's vehicle treatment, suggesting that E2 might prevent the impairing effect of TMX on the consolidation and retrieval of reward-related memory of morphine. This memory-enhancing effect of E2 was in a dose-dependant manner. The present findings suggest that TMX may affect morphine-induced contextual memory due in part to actions at ER. On the other hand, s.c. administration of TMX failed to completely block the significant enhancing effect of systemic E2 on CIP in place preference task. In this line, Frye and Rhodes (2002) showed that systemic TMX (10 mg/kg) failed to attenuate systemic or intrahippocampal E2's significant increase in crossover latencies in inhibitory avoidance paradigm. One possible explanation is that TMX is selective ER modulators and can have both agonistic and antagonistic actions at ER, depending on the target tissue. Furthermore, other experimental factors such as dose and timing of treatments should be considered. Our data showed that TMX 20 (mg/kg) had no impairing effect on morphine-induced CPP (data not shown), so we did not use higher doses of TMX in interaction experiments.

Previous studies reported that E2's mnemonic effects are time-dependent. Packard and Teather (1997) showed that post-training administration of E2 after 2 h is not effective on enhancing learning in the Morris water maze paradigm. It has been shown that a 1-h delay in administration of E2 after training can prevent its enhancing effects on the inhibitory avoidance task (Rhodes and Frye 2004). We showed that E2 administration 30 min before the second test for the CPP can enhance retrieval of reward-related memory. Together, these results suggest a rapid onset for E2's actions on these cognitive functions.

Our results suggest that administration of ER ligands, like E2 and TMX, can modify reward-related memory of morphine; TMX might impair this type of memory, at least in part, by acting on ER in the brain. However, further studies using more specific pharmacological tools or knockdown of ER are warranted to clarify the role of ER in this type of memory. An interaction between E2 and cholinergic function of the central nervous system has been shown; E2 affects cognitive performance by modulation of cholinergic function of the brain (Gibbs et al. 2004; Gibbs 2002). Further, an enhancing effect of E2 on NMDA receptors in the hippocampus has been reported previously; E2 might enhance cognitive performance through augmentation of NMDA function (El-Bakri et al. 2004). It is possible that TMX is acting through the cholinergic and/or glutamatergic neurotransmitter system to disrupt morphine-associated contextual memory.

In conclusion, these results demonstrate that tamoxifen appears to disrupt the consolidation and retrieval of morphine-associated contextual memory. Systemic E2 might reverse the impairing effect of TMX on this type of memory. The underlying mechanisms for these modulatory effects warrant further investigation.

Acknowledgments This study was supported by grant 6446 from the vice-chancellor for research of Tehran University of Medical Sciences to BE. We would like to thank Dr. M. A. Khoshnoodi for his helpful discussions and two anonymous reviewers for their constructive reviews.

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